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13. ABSTRACT *(Maximum 200 words)*

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The growth of E. coli is stimulated by exposure to nanopulses under moderate conditions - low electric feild strength (10kV/m), low pulse frequency (<10 kHz), and 120 min of exposure. E. coli is inexpensive to culture and perhaps the simplest organism known to man. Moreover, it has been studied in considerable depth, making it valuable for experiments aimed at determining the mechanism of observed bioeffects of nanopulse exposure. The finding that the growth of this species of bacteria is enchanced on exposure is potentially of considerable value to biotechnology. See Ayenapurapu et al .

The growth of a highly-malignant mouse mammary epithelial cell is stimulated by exposure to nanopulses under moderate conditions. These cells were chosen because they are a valuable model of apoptosis (programmed cell death) and are very familiar to Dr. Paul Sylvester, University of Louisiana at Monroe. The finding that these cells are stimulated on exposure is potentially of considerable value to tissue engineering. It could also be important for setting safety guidelines for exposure. This work is likely to result in at least one publication.

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4. Name of institution: Louisiana Tech University
5. Author of report: Dr Donald T. Haynie (PI)
6. List of manuscripts submitted/published since last progress report

   a. Conference presentations (presenter in italics)

      i. *R. Kompalli* and D. Haynie (2003) Effects of ultra-wideband electromagnetic pulses on erythrocytes, poster, ElectroMed 2003, San Antonio, TX, June 12.

      ii. S. Jones, *J. Banerjee*, and D. Haynie (2003) Activation of platelets by exposure to UWB, poster, ElectroMed 2003, San Antonio, TX, June 12.

      iii. *D. Haynie*, A. Lowrey and C. Glen McWright (2003) Mechanisms of cellular and sub-cellular bioeffects of UWB exposure, poster, Electromed2003, San Antonio, TX, June 12.

      iv. *A. Kalluri*, *R. Sunkam*, D. Haynie and L. Roemer (2003) UWB bioeffects: Electrical viewpoint, poster, Electromed2003, San Antonio, TX, June 12.

      v. W. Dai, D. Haynie, R. Nassar, N. Simicevic and *S. Su* (2003) Numerical simulation of electromagnetic fields in cells induced by nanopulses, poster, Electromed2003, San Antonio, TX, June 12.

      vi. *C. Ayenapurapu*, *M. Jalari*, and D.T. Haynie (2003) Effect of ultra-wideband pulses on bacteria, poster, Electromed2003, San Antonio, TX, June 12.

      vii. Sylvester, P.W., Haynie, D.T., Shah, S. and Briski, K.P. (2004) "Effects of Ultra-wideband (UWB) radiation on preneoplastic mammary epithelial cell proliferation *in*

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vitro," poster, *Proceedings of the American Association for Cancer Research*, vol. 45, abstract 197.

viii. *Sunkam, Ramana K.*, Selmic, Rastko R., Haynie, Donald T., and Hill, Jeremiah S. (2004) Solid-State Nanopulse Generator: Application in Ultra-wideband Bioeffects Research, oral presentation, IEEE Southeast Conference 2004.

ix. Haynie, D.T. (2004) "Low-field nanopulse bioeffects: models and mechanisms," oral presentation, annual Bioelectromangetics Society meeting, Washington, D.C., June.

x. Dorsey, W.C., Roane, L., and Haynie, D.T. (2004) "Induced mitogenic activity in AML-12 mouse hepatocytes exposed to ultra-wideband electromagnetic pulses," accepted poster abstract, First International Symposium on Recent Advances in Environmental Health Research, Jackson, MS, 20th-21st September.

b. Journal articles

i. Donald T. Haynie, Weizhong Dai, Steven A. Jones, Akundi N. Murty, Sateela Naidu, Raja Nassar, Louis Roemer, Rastko Selmic, Neven Simicevic, Paul W. Sylvester, Matthew F. Ware, Steven P. Wells, C. Mouli Ayenapurapu, Jayee Banerjee, Madan Jalari, Anil Kalluri, Rajesh Kompalli, Sumit Shah, Ramana K. Sunkam, J.D. Canfield, Robert Fox, and Jeremy Hill (2005) Establishment of a Bioelectromagnetics Research Facility: Mechanisms of Nanopulse Bioeffects, in preparation.

ii. Su, S., Dai, W., Haynie, D.T., Nassar, R. and Simicevic, N. (2004) Numerical Simulation of Nanopulse Penetration of Biological Matter Using the z-Transform, *Journal of Mathematical Modelling and Algorithms*, special issue on "Computational Science and Applications," in press.

iii. Su, S., Dai, W., Haynie, D.T. and Simicevic, N. (2004) Use of the z-Transform to Investigate Nanopulse Penetration of Biological Matter, *Bioelectromagnetics*, in press.

iv. Sunkam, Ramana K., Hill, Jeremy S., Selmic, Rastko R., and Haynie, Donald T. (2005) Solid-State Nanopulse

Generator: Application in Ultra-wideband Bioeffects Research, *Rev. Sci. Instrum.*, in press.

v. Dorsey, W.C., Ford, B.D., Roane, L., Haynie, D.T., Tchounwou, P.B. (2005) "Induced mitogenic activity in AML-12 mouse hepatocytes exposed to low-dose ultra-wideband electromagnetic radiation, accepted poster abstract," accepted paper for conference proceedings, First International Symposium on Recent Advances in Environmental Health Research, Jackson, MS, 20th-21st September.

vi. Sylvester, P.W., Shah, S.J., Haynie, D.T. and Briski, K.P. (2005) "Effects of ultra-wideband pulses on preneoplastic mammary epithelial cells proliferation," in preparation.

vii. Ayenapurapu, Chandra M. and Haynie, D.T. (2005) Effect of nanopulses on growth of *Escherichia coli*, under revision for publication.

viii. Banerjee, J., Kompalli, R., Haynie, D.T. and Jones, S.A. (2005) Effects of low-electric field strength nanopulses on non-proliferative cells, under revision for publication.

ix. Simicevic, N. and Haynie, D.T. (2005) "FDTD Simulation of Exposure of Biological Material to Electromagnetic Nanopulses," *Phys. Med. Biol.*, **50**:347-360.

x. Several other manuscripts in preparation, on effect of nanopulses on electron transfer, effect of nanopulses on *C. elegans*, *etc.*

7. Author of report: Dr Donald T. Haynie (PI)

8. Inventions, patents, discoveries

   a. Reports of invention

      i. R. Sunkam, R. Selmic, and D. Haynie (2003) Low-cost, fast-risetime, nanosecond-long electromagnetic pulse generator.

   b. Patents – none as yet. Please contact Dr L. Guice, vice-present of Research and Development at Louisiana Tech, to enquire about whether the University will seek to patent the technology described in the report of invention.

c. Discoveries – see "Key Findings"

9. Collaborators/consultants

a. Andrei Pakhomov, McKesson BioServices, Brooks City Base – This collaboration was initiated in June 2003. The intent was serious and the future seemed promising. The main purpose of the proposed work was to corroborate the findings on *E. coli*, and to strengthen ties with Brooks. A Louisiana Tech graduate student was to visit San Antonio. Unfortunately, a serious automobile accident on Interstate 20 involving several students working on this research (Mouli Ayenapurapu, Madan Jalari, and others) made it impossible to follow through on this intention.

b. Eric Aamodt, Louisiana State University Health Sciences Center, Shreveport – This collaboration was initiated in July 2003. Extensive data on nematode worm exposure have been obtained. An NIH proposal has been submitted and a manuscript will be ready shortly.

c. Co-investigators since awarding of contract: Matthew F. Ware, Grambling State University, Naidu Seetala, Grambling State University, Paul W. Sylvester, University of Louisiana at Monroe.

10. Honors or awards

a. $300, "Million dollar club," LA Tech, D. Haynie, for external grant income in one year totaling over $1 m.

b. $600, "Grant in aid of research," Sigma Xi, J. Hill, for undergraduate research.

11. Key Findings/results/accomplishments

a. Theoretical analysis suggests that the nanopulse experiments we have carried out (typically 20 kV/m for 1-4 h of exposure) do not cause sample heating. See Simicevic and Haynie (2005).

b. The growth of *E. coli* is stimulated by exposure to nanopulses under moderate conditions – low electric field strength (~10 kV/m), low pulse frequency (<10 kHz), and 120 min of expsoure. *E. coli* is inexpensive to culture and perhaps the simplest organism known to man. Moreover, it has been studied in considerable depth, making it valuable for experiments aimed at determining the mechanism of observed bioeffects of nanopulse exposure. The finding that the growth of this species of bacteria is enhanced on exposure is potentially of considerable value to biotechnology. See Ayenapurapu et al.

c. The growth of a highly-malignant mouse mammary epithelial cell line is stimulated by exposure to nanopulses under moderate conditions. These cells were chosen because they are a valuable model of apoptosis (programmed cell death) and are very familiar to Dr Paul Sylvester, University of Louisiana at Monroe (30 miles east of Louisiana Tech). The finding that these cells are stimulated on exposure is potentially of considerable value to tissue engineering. It could also be important for setting safety guidelines for exposure. This work is likely to result in at least one publication. See Sylvester et al.

d. The development of *C. elegans*, a nematode worm, is influenced by nanopulse exposure under moderate conditions (20 kV/m, <75 min exposure). These animals were selected for study because they are inexpensive to culture, no special permit is required to do research on them, they are arguably the most thoroughly studied animals of all, they are remarkably similar to humans in notable respects, and they are the chief object of study of Eric Aamodt, University of Louisiana Health Sciences Center, Shreveport (70 miles west of Louisiana Tech). We have found that nematode worms lay more eggs but that more of the eggs do not hatch on nanopulse exposure, relative to control. It is unclear whether eggs themselves or, possibly, sperm are affected. The result is unlikely to depend on heating. There are three reasons for this: 1) on theoretical grounds no sample heating is expected for nanopulses of such low electric field strength; 2) temperature measurements show that there is no sample heating of aqueous samples; 3) a heat shock strain of worms expressing green fluorescent protein shows no increase in fluorescence relative to control on nanopulse exposure. The effects of nanopulses on fertility, then, would appear to be a direct effect of exposure. It is by no means clear, however, which cellular, supercellular, or subcellular structures might be affected. A manuscript on results obtained thus far is in preparation.

e. Platelets and red blood cells seem not to be affected by nanopulse exposure under mild conditions. These models were chosen because they are inexpensive to obtain and prepare. Moreover, platelets show a number of clear markers of activation, which can be stimulated by various types of stress, and the biophysical properties of red blood cells have been studied extensively, important to elucidation of mechanism. This work is likely to result in at least one publication. See Banerjee et al.

f. Solid-state nanosecond-long pulse generators can be built at modest cost. Depending on the future of nanopulse bioeffects research, this could be significant for technology development and research in biomedicine. See Sunkam et al. (2005).

g. Electron transfer. We have found that horseradish peroxidase, a well-known enzyme of biotechnological interest, shows an increased rate of inactivation in aqueous solution on nanopulse exposure, relative to control. The effect is mitigated in the presence of vitamin C or vitamin E, known free radical scavengers. It is unclear, however, why nanopulses might produce free radicals, because the energy density above 1 GHz is relatively low. One possibility is that nanopulses somehow interfere with the oxidation state of iron in heme, as a consequence of this would be alteration of enzyme activity.

h. None of the research achievements described here would have been possible without the development of a test facility. This was done virtually from scratch. The most significant aspects of building the facility have been: developing a team of researchers at the levels of faculty, post-doc, graduate student, and undergraduate student; defining an overall research strategy; assessing, procuring, and setting up large items of equipment; directing the research; and maintaining momentum. See Haynie et al. (in preparation).

12. Transitions/technology transfers

Transfer of the pulser work has not actually occurred as yet.